Anti-Cytokine Therapies in Response to Systemic Infection

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In the past 5 y, the 28 d mortality in patients with sepsis syndrome has decreased somewhat but still ranges from 30% to 40%; mortality in those patients with septic shock and multiple organ failure is higher. This high mortality is observed despite intensive care units that deliver hemodynamic, metabolic, ventilatory, and renal support. Clearly some patients survive the ordeal but it remains frustrating not being able to stop the downhill course leading to multiple organ failure and death in these patients. New therapies have been sought and tested, including those preventing the biologic activity of two pro-inflammatory cytokines, interleukin-1 (IL-1) and tumor necrosis factor (TNF). Based on animal studies, anti-TNF and IL-1 therapy has been used to "rescue" the patient who continues to deteriorate in the face of considerable support efforts. Unfortunately, these anticytokine therapies have not dramatically reduced 28 d mortality in double-blind, placebo-controlled trials involving nearly 10 000 patients, although there is a consistent but statistically nonsignificant decrease in mortality associated with anticytokine therapies. On the other hand, the same anti-TNF and IL-1-based therapies have made a dramatic improvement in the local inflammation and progression of rheumatoid arthritis. It appears that systemic inflammation of sepsis requires more than anticytokine monotherapy to significantly reduce mortality. ***Key words: interferon-γ/interleukin-1/septic shock/tumor necrosis factor. Journal of Investigative Dermatology Symposium Proceedings 6:244–250, 2001***

Cytokines are small, nonstructural proteins with molecular weights ranging from 8 to 40 000 Da. Originally called lymphokines and monokines to indicate their cellular sources, it became clear that the term "cytokine" is the best description as nearly all nucleated cells are capable of synthesis of these proteins and, in turn, respond to them. There is no amino acid sequence motif or three-dimensional structure that links cytokines; rather, their biologic activities allow us to group them into different classes. For the most part, cytokines are primarily involved in host responses to disease or infection and any involvement with homeostatic mechanisms has been less than dramatic.

CYTOKINE RESPONSES TO INFECTION AND INFLAMMATION

There are presently 18 cytokines with the name "interleukin". Other cytokines have retained their original biologic description such as "tumor necrosis factor". Another way to look at some cytokines is their role in infection and/or inflammation. Hence some cytokines clearly promote inflammation and are called pro-inflammatory cytokines, whereas others suppress the activity of pro-inflammatory cytokines and are called anti-inflammatory cytokines. For example, interleukin (IL)-4, IL-10, and IL-13 are potent activators of B-lymphocytes; however, IL-4, IL-10, and IL-13 are also potent anti-inflammatory agents. They are anti-inflammatory cytokines by virtue of their ability to suppress genes for pro-inflammatory cytokines such as IL-1, tumor necrosis factor (TNF), and the chemokines.

IFN-γ is another example of the pleiotropic nature of cytokines. Like IFN-α and IFN-β, IFN-γ possesses antiviral activity. IFN-γ is also an activator of the pathway that leads to cytotoxic T cells; however, IFN-γ is considered a pro-inflammatory cytokine because it augments TNF activity and induces nitric oxide (NO). Therefore, listing cytokines in various categories should be done with an open mind in that depending upon the biologic process, any cytokine may function differentially.

THE CONCEPT OF PRO- AND ANTI-INFLAMMATORY CYTOKINES

The concept that some cytokines function primarily to induce inflammation whereas others suppress inflammation is fundamental to cytokine biology and also to clinical medicine. The concept is based on the genes coding for the synthesis of small mediator molecules that are upregulated during inflammation. For example, genes that are pro-inflammatory are phospholipase A2 type-II, cyclooxygenase-2 (COX-2), and inducible NO synthase (iNOS). These genes code for enzymes increasing the synthesis of platelet activating factor and leukotrienes, prostanoids, and NO. Another class of genes that are pro-inflammatory are chemokines, small peptides (8000 Da) that facilitate the passage of leukocytes from the circulation into the tissues. The prototypic chemokine is neutrophils chemoattractant IL-8. IL-8 also activates neutrophils to degranulate and cause tissue damage. IL-1 and TNF are inducers of endothelial adhesion molecules, which are essential for the adhesion of leukocytes to the endothelial surface prior to emigra-

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Abbreviations: ARDS, acute respiratory distress syndrome; COX-2, cyclooxygenase type 2; ICE, IL-1β converting enzyme; IL-1R, IL-1 receptor; IL-1Ra, IL-1 receptor antagonist; NFκB, nuclear factor B; NO, nitric oxide; PGE2, prostaglandin E2; TNFR, TNF receptor; TRADD, TNF receptor associated death domains.

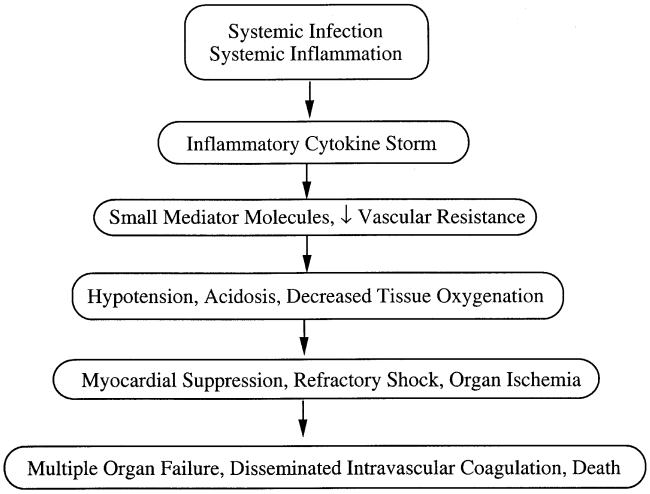

Figure 1. Physiologic events associated with the development of septic shock.

tion into the tissues. Taken together, pro-inflammatory cytokine-mediated inflammation is a cascade of gene products usually not produced in health. What triggers the expression of these genes? Although inflammatory products such as endotoxins do, the cytokines IL-1 and TNF (and in some cases IFN-γ) are particularly effective in stimulating the expression of these genes. Moreover, IL-1 and TNF act synergistically in this process. Whether induced by a infection, trauma, ischemia, immune-activated T cells, or toxins, IL-1 and TNF initiate the cascade of inflammatory mediators by targeting the endothelium. **Figure 1** illustrates the inflammatory cascade triggered by IL-1 and TNF.

On the other hand, anti-inflammatory cytokines block this process or at least suppress the intensity of the cascade. Cytokines such as IL-4, IL-10, IL-13, and TGF-β suppress the production of IL-1, TNF, chemokines such as IL-8, and vascular adhesion molecules. Therefore, a "balance" between the effects of pro- and anti-inflammatory cytokines is thought to determine the outcome of disease, whether short or long-term. In fact, some studies have data suggesting that susceptibility to disease is genetically determined by the balance or expression of either pro- or anti-inflammatory cytokines; however, gene linkage studies are often difficult to interpret. Nevertheless, deletion of the IL-10 gene in mice results in the spontaneous development of a fatal inflammatory bowel disease. Deletion of the TGF-β1 gene also results in a spontaneous inflammatory disease. In mice deficient in IL-1 receptor antagonist (IL-1Ra), spontaneous disease nearly identical to rheumatoid arthritis is observed.

IL-1 AND TNF

Synergism of IL-1 and TNF is a commonly reported phenomenon. Clearly, both cytokines are being produced at sites of local inflammation and hence the net effect should be considered when making correlations between cytokine levels and severity of disease. There is also synergism between IL-1 and bradykinin as well as IL-1 or TNF and mesenchymal growth factors. Most relevant to pain is the increase in PGE2 stimulated by IL-1 or the combination of IL-1 and TNF. IL-1 also lowers the threshold to pain primarily by increasing PGE2 synthesis (Schweizer *et al*, 1988). **Table I** summarizes the synergism of IL-1 and TNF.

Humans injected with IL-1 experience fever, headache, myalgias, and arthralgias, each of which is reduced by coadministration of cyclooxygenase inhibitors.[1] One of the more universal

[1]Smith JW, Urba WJ, Curti BD, *et al*: Phase II trial of interleukin-1 alpha in combination with indomethacin in melanoma patients. *Proc Am Soc Clin Oncol* 10:293, 1991 (abstr.)

Table I. Synergistic activities of IL-1 and TNF

Hemodynamic shock and lactic acidosis in rabbits
Radioprotection
Generation of Shwartzman reaction
Luteal cell $PGF_{2\alpha}$ synthesis
PGE_2 synthesis in fibroblasts
Galactosamine-induced hepatotoxicity
Sickness behavior in mice
Circulating nitric oxide and hypoglycemia in malaria
Nerve growth factor synthesis from fibroblasts
Insulin release and islet beta cell death
Insulin resistance
Loss of lean body mass
IL-8 (and other chemokine) synthesis

activities of IL-1 is the induction of gene expression for type-II phospholipase A2 and COX-2. IL-1 induces transcription of COX-2 and seems to have little effect on increased production of COX-1. Moreover, once triggered, COX-2 production is elevated for several hours and large amounts of PGE2 are produced in cells stimulated with IL-1. Therefore, it comes as no surprise that many biologic activities of IL-1 are actually due to increased PGE2 production. There appears to be selectivity in cyclooxygenase inhibitors in that some nonsteroidal anti-inflammatory agents are better inhibitors of COX-2 rather than COX-1. Similar to COX-2 induction, IL-1 preferentially stimulates new transcripts for the inducible type-II form of PLA2, which cleaves the fatty acid in the number 2 position of cell membrane phospholipids. In most cases, this is arachidonic acid. The release of arachidonic acid is the rate-limiting step in the synthesis of prostaglandins and leukotrienes. IL-1 also stimulates increased leukotriene synthesis in many cells.

HOW DOES IL-1 DIFFER FROM TNF IN ACTIVATING CELLS?

From the above descriptions of IL-1R and IL-1 signal transduction, many of these pathways are shared with TNF. Although the receptors for TNF and IL-1 are clearly different, the postreceptor events are amazingly similar. Thus, the finding that IL-1 and TNF activate the same portfolio of genes is not surprising; however, given the same cell and given the same array of activated genes, IL-1 does not result in programmed cell death, whereas TNF does. This can be seen in TNF responsive fibroblast in which IL-1 and TNF induce IL-8 but in the presence of actinomycin C or cycloheximide, TNF induces classical apoptosis but IL-1 does not. IL-1 will often synergize with TNF for NO induction and under those conditions, NO mediates cell death. The best example of this can be found in the insulin-producing β cells in the islets of Langerhans in the pancreas (Reimers *et al*, 1994). Unlike IL-1, the receptors for TNF are homodimers and trimers and hence the recruitment of kinases is somewhat different; however, the cytosolic domain of the TNF p55 receptor contains a "death domain" that recruits intracellular molecules involved with initiating programmed cell death (Boldin *et al*, 1995). There is no comparable "death domain" in the cytoplasmic domains of either the IL-1RI or the IL-1R-AcP.

There are two receptors for TNF, the p55 receptor and the p75 receptor (Engelmann *et al*, 1990). Although TNF binds and triggers both receptors, the cytosolic domains of these receptors recruit different proteins that transduce the TNF signal further. In once case, the p55 receptor cytosolic domain is linked to pathways of cell death, whereas the p75 is not. Both receptors, however, result in the translocation of the nuclear factor B (NFκB) to the nucleus, where it binds to the promoter regions of a variety of genes. These gene products are often the same as those triggered by IL-1, which also results in translocation of NFκB to the nucleus. The difference, however, is that the cytosolic domains of the p55 TNFR are unique in their ability to activate intracellular signals leading to programmed cell death (also called apoptosis). The p55 TNFR has

the so-called ''death domain'' and recruits a protein called MORT-1. Also involved in this process are a family of intracellular proteins that become activated and are called TRAF for TNF receptor associated factors. Presently there are six or perhaps eight TRAF. The p55 cytosolic domains also recruit the family of intracellular proteins called TNF receptor associated death domains (TRADD). Overexpression of TRADD results in cell death. It also leads to activation of NFκB. TRADD also lead to activation of the caspase family of intracellular cysteine proteases. Although caspase-1 (also known as the IL-1β converting enzyme, ICE) is important for processing the precursors for proIL-1β and proIL-18, other members of this family are also part of the TNF cell death signal pathway.

One interesting aspect of the biology of TNF in the brain is the ability of TNF to both protect neurons as well as to initiate their self destruction. Both pathways involve activation of NFκB (Hunter *et al*, 1997). In general, the state of the cell (cell cycle) may help explain why activation of NFκB can be associated with both protection of cell death as well as apoptosis. One is reminded that activation of NFκB leads most often to new protein synthesis; some proteins from this process are clearly inducing cell proliferation whereas others induce cell death.

THE PHYSIOLOGIC CATASTROPHE OF SEPTIC SHOCK AND THE CONCEPT OF RESCUE

The diagnosis of sepsis syndrome or septic shock is based on a constellation of physiologic, metabolic, and hematologic abnormalities most commonly occurring in patients with a known infection. In most cases, the patient is already hospitalized with an antecedent illness or has experienced recent surgery. Many patients, but not all, are being treated with appropriate antibiotics for a suspected infection. Approximately 25% of patients with sepsis syndrome are in hemodynamic shock at the time of presentation (Sands *et al*, 1997). Characteristic of septic shock, the hypotension is unresponsive to rapid fluid replacement. In most circumstances, when blood pressure falls (a 25%–35% reduction), 500 ml of saline infusion is rapidly administered. If there is no response with an increase in mean arterial pressure, a diagnosis of refractory hemodynamic shock is made. In the context of an on-going infection or suspected new infection, the patient is thought to be in septic shock.

If the hypotension is not corrected, progressive reduction in organ perfusion and increasing acidosis leads to tissue hypoxia and results in organ failure. Although the hypotension is often initially treated with vasopressor drugs and antibiotics are either changed or additional antibiotics used, a downhill course can rapidly take place, resulting in death. In some patients, this rapid downhill course can be very dramatic and disseminated intravascular coagulation may develop. This physiologic cascade is illustrated in **Fig 1**.

There is little question that the major advance in treating patients with septic shock has been the availability of broad spectrum antibiotics. In fact, the sooner broad spectrum antibiotics are administered the lower the mortality rate (Dunn, 1994). The testing of nonantibiotic-based, novel therapies is based on 50 y of research on how microorganisms trigger the cascade of events in septic shock. Although microbial products such as endotoxins are still targets for therapy, a fundamental concept is that the constellation of abnormalities in these patients results from the patient's own response to the infection (or in some cases their response to massive trauma and blood loss). Initially, activation of complement was considered causal, particularly the fifth component of complement that is a potent neutrophil activator and produces a capillary leak syndrome. The release of platelet-activating factor (PAF) was also thought to be responsible, particularly as PAF is a potent hypotensive agent. Using specific inhibitors of PAF, animals given lethal bacterial toxins survive. Similar results were obtained when cyclooxygenase inhibitors were administered to animals challenged with lethal amounts of endotoxin or bacteria; hence those experiments implicated cylooxygenase products as a contributing cause to septic shock. Numerous animal studies also demonstrated the protective effects of cortiocosteroids, and several large clinical trials using these potent anti-inflammatory agents were undertaken. Clinical trials of PAF antagonists, cyclooxygenase inhibitors, bradykinin antagonists, and corticosteroids have each failed to reduce significantly the 28 d mortality in septic shock patients.

CYTOKINES IN PATHOGENESIS OF SEPSIS AND SEPTIC SHOCK

The field entered a new era when it was shown that neutralizing antibodies to the inflammatory cytokine TNF prevented death in mice (Beutler *et al* 1985), rabbits (Mathison *et al* 1988), or baboons (Tracey *et al*, 1987) following a lethal injection of *E. coli* or endotoxin. Previously it had been shown that in the absence of any infection, high doses of TNF in animals induced circulatory collapse and organ necrosis (Tracey *et al*, 1987), which were very similar to those observed in humans with septic shock (reviewed in Beutler and Cerami, 1987). Similar results were observed with high doses of IL-1 in animals (Bertini *et al* 1988). Injecting a combination of low doses of IL-1 plus TNF revealed that these two cytokines acted synergistically in inducing a shock-like state (Okusawa *et al*, 1988). Similar to neutralizing TNF activity, blocking IL-1 receptors were also effective in preventing death in animal models of lethal bacteremia or endotoxemia (Ohlsson *et al*, 1990; Wakabayashi *et al*, 1991). Because TNF induces IL-1 and IL-1 induces TNF, synergism between the two cytokines takes place. For the patient without overt infection, e.g., multiple trauma, the preclinical data demonstrated that the systemic injection of either IL-1 or TNF into experimental animals induced physiologic, hematologic, and pathologic changes that were nearly identical to those observed during bacteremia or multiple trauma.

Animal studies were confirmed when humans were injected with either IL-1 or TNF. The most impressive physiologic event following the intravenous injection of either cytokine in humans is the fall in blood pressure (van der Poll *et al* 1990). Frank hypotension has been reported with doses of IL-1 or TNF as low as 50 ng per kg (Chapman *et al*, 1987; Smith *et al*, 1993). The hypotension is concentration dependent and despite a short plasma half-life of less than 10 min, the biologic consequences can be observed for days. In studies in which IL-1 was administered as adjunct therapy for bone marrow transplant recovery, nearly all patients required vasopressor therapy (Smith *et al*, 1992, 1993). In the case of TNF, an increase in the coagulation parameters and an early leukopenia occurred (van der Poll *et al*, 1990, 1991).

THE RATIONALE FOR ANTI-CYTOKINE THERAPY

The biologic basis for the development of a shock-like state following systemic IL-1 or TNF has been established at the molecular level. Both cytokines activate the transcription of genes which increase the production of small, potent mediator molecules. For example, IL-1 and TNF increase gene expression and synthesis for phospholipase A2 type II leading to increased PAF synthesis. Similarly an increase in COX-2 by IL-1 and TNF results in elevated levels of PGE2 (reviewed in Dinarello, 1996). On a molar basis, NO is perhaps the most potent vasodilator and is thought to be primarily responsible for the hypotension and myocardial suppression in septic shock (Moncada *et al* 1991). Whereas constitutive NO production is part of homeostasis, increased production of NO in inflammation takes place when NO is the product of inducible NO synthase (Fang, 1997). IL-1, TNF, and IFN-γ, particularly the combination of the three, activate gene expression and synthesis of inducible NO synthase; however, agents that are competitive inhibitors of arginine will reduce synthesis of both forms of NO.

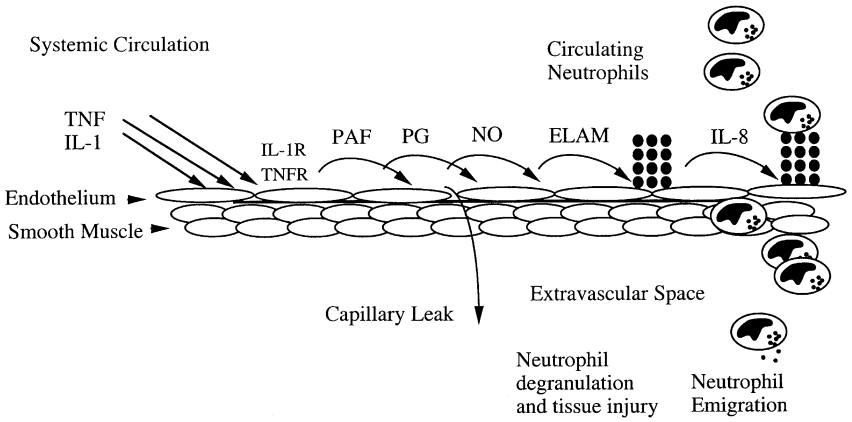

Figure 2. Effect of IL-1 and TNF on endothelium. TNF and IL-1 activate endothelial cells and trigger the cascade of pro-inflammatory small molecule mediators. Increased gene expression for phospholipase A2 type II, COX-2, and iNOS results in elevated production of their products, PAF, PGE2, and NO. Alone or in combination, these mediators decrease the tone of vascular smooth muscle and systemic vascular resistance takes place. IL-1 and TNF also cause increased capillary leak. The upregulation of endothelial leukocyte adhesion molecules results in adherence of circulating neutrophils to the endothelium and increased production of chemokines such as IL-8 facilitates the emigration of neutrophils into the tissues. Chemokines also activate degranulation of neutrophils. Activated neutrophils lead to tissue destruction, particularly in the lung.

META-ANALYSIS OF SEPTIC SHOCK INTERVENTION TRIALS

Several double-blinded, placebo-controlled trials were carried out in order to neutralize TNF activity with either monoclonal anti-TNF or soluble TNF receptors and to block IL-1 activity with the IL-1 receptor antagonist (Fisher *et al*, 1994a, b; Abraham *et al*, 1995, 1997). With the exception of one trial using the soluble TNFR p75:Fc (Fisher *et al*, 1996), there was a small but consistent improvement in 28 all-cause mortality. The results of these trials in the overall sepsis syndrome population have been disappointing. The most recent and largest trial in 105 medical centers recruited 1879 patients with septic shock for randomization to placebo or neutralizing monoclonal antibodies to TNF. Improved 28 d survival of 2.5% was observed (40.3% for anti-TNF *versus* 42.8% for the placebo) (Abraham *et al*, 1998). In addition, the results of a large trial using a construction of two chains of the p55 TNF extracellular receptor linked to the Fc domain of human IgG has again revealed no significant reduction in 28 d mortality.

A second Phase III trial in 91 academic centers in North America and in Europe was initiated intending to randomize 1300 patients to either placebo or IL-1Ra. The IL-1Ra was administered as an intravenous bolus injection of 100 mg followed by 3 d of constant infusion of 2.0 mg per kg per h. The primary endpoint was survival time in patients with end-organ dysfunction and/or shock at the time of entry. There were 512 patients who met these entry criteria. Another 184 were entered into the study but had secondary endpoints such as shock. A mid-trial analysis was undertaken after 696 patients had been enrolled. The study was terminated during an interim analysis because a reduction in overall 28 d mortality would not likely reach statistical significance. Analysis of the entire 696 patients was made. The 28 d mortality from all causes in the placebo arm was 36.4% and 33.1% in the patients receiving IL-1Ra, a 9% reduction in mortality, $p = 0.36$. The patient groups were well matched in that 52.9% of the placebo patients and 50.9% of the IL-1Ra group were in shock at the time of study entry. There was no excess mortality in patients receiving IL-1Ra (Opal *et al*, 1997).

A meta-analysis was performed (Zeni *et al*, 1997) in order to examine the outcomes in terms of safety and efficacy of the many trials in septic shock patients. The analysis included 39 trials conducted over the past 30 y. There were 20 trials of non-glucocorticoids such as IL-1R antagonist, antibodies to TNF and bradykinin and PAF antagonists. There were 10 trials for testing of antiendotoxin antibodies. There were several conclusions to the analysis: (i) the mortality of the control arm (placebo) for the entire group of 39 trials was consistently 35%–40%; (ii) high-dose glucocorticoids showed a harmful effect on survival; (iii) anti-mediator trials resulted in a small but significant survival benefit; and (iv) antiendotoxin trials showed no effect. It is important to note that with the exception of a single trial (Fisher *et al*, 1996), the anticytokine therapy trials did not increase mortality but rather 28 d mortality was decreased. With anticytokine therapy, the decrease in mortality was small (2%–5%). This latter result is in contrast to predictions that blocking the biologic effects of IL-1 or TNF would reduce host defense and increase mortality. In these highly vulnerable patients with severe infection, blocking IL-1 activity or neutralizing TNF had no harmful effect.

OTHER THERAPIES

In mice, treatment with neutralizing antibodies to IFN-γ, are protective against the lethal effect of endotoxemia (Heremans *et al*, 1990). Similar data have been reported for mice lacking the receptor for IFN-γ (Huang *et al*, 1993). In mice deficient in the IL-1β converting enzyme, there is decreased circulating IFN-γ and these mice are also resistant to endotoxin-mediated death (Kuida *et al*, 1995; Li *et al*, 1995). IFN-γ is a potent macrophage activator and increases the production of and response to TNF; however, in contrast to a causative role of IFN-γ in death in mice, the administration of IFN-γ to several thousand patients with burns, infections, or cancer is not associated with increased death of development of shock. In fact, patients with septic shock appear to benefit from treatment with IFN-γ (Doecke *et al*, 1997). The rationale for using IFN-γ in patients with septic shock is based on the observation that some patients exhibit signs of decreased macrophage and T cell function during sepsis and that IFN-γ restores these immunosuppressed patients. These observations on IFN-γ require a double-blind, placebo-controlled trial using greater numbers of patients. In a prospective study of 184 patients undergoing gastrointestinal surgery, depressed monocyte IL-12 production prior to the operation was selectively correlated to the severity of postoperative sepsis (Hensler *et al*, 1998).

Other therapeutic approaches in septic patients target the chemokines and adhesion molecules. Although administration of large doses of IL-8 to primates does not result in hypotension, neutralizing antibodies to IL-8 in models of inflammation reduce neutrophil infiltration in the lung, joint, kidney, skin, and myocardium (Harada *et al*, 1996). In particular, anti-IL-8 reduces neutrophil accumulation into the lung and myocardium following ischemia-reperfusion injury. If tested in humans, anti-IL-8 therapy would most likely be used in patients with acute respiratory distress syndrome (ARDS). IL-8 is increased in bronchoalveolar lavage specimens from patients with ARDS and, in most patients at risk for ARDS, elevated bronchoalveolar lavage IL-8 levels have been predictive of the subsequent development of ARDS (Donnelly *et al*, 1993). Blocking IL-8 reduces the entrance of neutrophils into inflammatory sites (Harada *et al*, 1996). Although IL-8 is a sensible target in septic shock patients, particularly in halting the progress of ARDS, the production of IL-8 (and other chemokines) is markedly reduced by the combination of anti-IL-1 and anti-TNF agents. As shown in **Fig 2**, blocking the biologic activities of IL-1 and TNF is

an upstream strategy for reducing the cascade of secondary mediators of systemic inflammation.

A CLOSER LOOK AT THE PATIENT POPULATION ENTERING SEPSIS TRIALS

Nearly all of the clinical trials investigating new therapies for sepsis have used a broad definition of the ''sepsis syndrome'', based on clinical criteria including the presence of tachycardia, hyper- or hypothermia, and elevated or decreased peripheral white blood cell counts. These are coupled with the presence of organ system dysfunction, such as lactic acidosis, disseminated intravascular coagulation, hypoxemia, hypotension, or decreased urine output. These entry criteria have permitted patients with a wide range of underlying illnesses and sources of infection to be treated. There is little question that these patients form a very heterogeneous population. For example, patients with clinical evidence of urinary tract, respiratory, or intra-abdominal infection, with underlying illnesses as diverse as cancer, autoimmune disorders, chronic renal failure, and diabetes, were included in the clinical studies with IL-1Ra or anti-TNF therapies. Infections due to Gram-positive, Gram-negative, or fungal organisms are included. American and European studies also reflect the dilemma of patient heterogeneity despite identical entry criteria and drugs (Abraham *et al*, 1995; Cohen and Carlet, 1996). The heterogeneity of patients enrolled in sepsis studies can be contrasted to the defined groups used in studies of IL-1Ra or anti-TNF therapies in rheumatoid arthritis or inflammatory bowel disease. In these latter two diseases, the underlying mechanism of the disease process is the same for all patients.

In animal models of infection, cytokine release is dependent on the source and type of infection. For example, anti-TNF therapies in murine models appear to work best for bacteremia and have poor or no efficacy in intraperitoneal infections. Yet, only a minority of patients in the trials examining anti-TNF therapies were bacteremic. Furthermore, positive microbial cultures are not reported in approximately one-third of the patients enrolled into these trials, despite clinical evidence or suspicion of infection. Therefore, the actual nature of the infectious process in these patients remains unclear. In one trial of IL-1Ra, there was a clear survival benefit over the placebo in bacteremic patients compared with non-bacteremic patients (Fisher *et al*, 1996).

In one study, cytokine levels were used as an entry criterion. In that trial employing murine anti-TNF Fab2' fragments, a single circulating IL-6 level greater than 1000 pg per ml was used to identify a target patient group for this therapy (Reinhart *et al*, 1996). Although IL-6 levels consistently correlate with disease severity in most patients with sepsis, patients with infectious and noninfectious diseases show remarkably variable circulating and tissue cytokine levels and a single measurement can be misleading. Because circulating levels of endogenously produced IL-1Ra and TNF soluble receptors are elevated in sepsis, the net biologically active IL-1 or TNF remains unclear. IL-6 levels may be a better marker of the net biologic effect of IL-1 plus TNF. It would appear more useful to identify for enrollment those patients with increased generation of biologically active cytokine(s), which is the target of blocking therapy. Unfortunately, such identification is difficult because TNF and IL-1 are released at their greatest concentrations at the tissue sites, and rarely do the circulating levels correlate with the levels of local production. Therefore, clinical criteria will probably be required to identify patients with increased tissue IL-1 and TNF. These patients appear to be those with a rapidly occurring onset of organ system dysfunction secondary to infection, without major underlying and preexistent medical problems. An example would be the young patient with acute onset of meningococcemia, in whom circulating and tissue levels of TNF-α are dramatically elevated. In this setting, the greatest survival benefit from anticytokine therapy would be expected when the agent(s) is given very soon after diagnosis and would be observed in the period immediately following therapy, as a direct result of reversal of pro-inflammatory cytokine-driven organ dysfunction.

IS THERE A GENETIC PREDISPOSITION TO SURVIVING SEPTIC SHOCK?

Targeted deletion of the IL-1Ra gene in mice (also known as IL-1Ra knockout mice) has resulted in a phenotype highly vulnerable to endotoxin-induced lethality, whereas mice overexpressing IL-1Ra appear to be protected against lethal endotoxemia (Hirsch *et al*, 1996). These latter experiments suggest that endogenous levels of IL-1Ra may contribute to disease outcome, at least in the case of septic shock. In addition, in mice deficient in IL-1Ra, spontaneous rheumatoid arthritis-like disease develops (Horai *et al*, 2000). Also, mice deficient in IL-1Ra develop a lethal arteritis (Nicklin *et al*, 2000).

The working hypothesis is that those individuals with the genetic make-up to produce large amounts of IL-1Ra when septic, are afforded a greater level of protection than another subject producing lower levels. The parallel working hypothesis is that those individuals with the genetic make-up to produce less bioactive IL-1β when septic, are less likely to die during septic shock compared with those subjects producing larger amounts of IL-1β. Although the ultimate proof of these two hypotheses is to measure the concentrations of IL-1Ra and IL-1β in patients surviving and compare those levels with those patients dying of septic shock, such measurements in the context of the acute setting are affected by nutritional and nongenetic mechanisms present at the time of infection. Hence, an investigation into the genetic determinants that may result in high or low IL-1Ra or IL-1β production should shed some light onto that question.

Messenger RNA does not cause disease. Even if the promoters for IL-1Ra and the cleavage site for IL-1β are genetically different, unless these differences result in different levels of the gene product, polymorphisms are of questionable importance to the outcome of disease. Nevertheless, there are lessons to be learned from examination of polymorphisms in cytokine genes. For example, persons homozygous for the TNFB2 allele of the NcoI site in the TNF locus are associated with nonsurvival in patients with severe sepsis (Stüber *et al*, 1996). These patients also have elevated TNF-α levels in the circulation and higher organ failure scores. Although there is increased mortality with the homozygous TNFB2 allele, there is no difference between the frequency of this polymorphism in the general population and the group of patients in the intensive care unit with a diagnosis of severe sepsis. It appears that if one develops severe sepsis, inheriting the TNFB2 allele makes one particularly at risk for death compared with heterozygotes or patients homozygous for the TNFB1 allele (Stüber *et al*, 1996).

Another study (Fang *et al*, 1999) examined two well-described genetic polymorphisms in the IL-1 family: the A1-5 allele in intron 2 of the IL-1Ra gene and the Taq1 site in exon 5 of the IL-1β gene. A comparison of the frequency of these alleles in 93 consecutive patients admitted to the surgical intensive care unit with 261 local blood donors in apparent health was made. The results were surprising in that there was a high frequency of the IL-1RaA2 allele in the cohort with severe sepsis compared with the healthy cohort ($p < 0.01$). Although there was no association with outcome (survival at 28 d), the conclusion of the study suggests that persons with this allele are more likely to find themselves in a surgical intensive care unit with severe sepsis that those without the allele. The internal control for this study was the lack of the Taq1 allele associated with this cohort of 93 patients compared with the population of 261 persons.

In addition, these studies confirmed that the TNFB2 allele was again associated with nonsurvival in this cohort; however, there was no linkage between these two polymorphisms in the study. Yet, in eight individuals who were born homozygous for both the IL-1RaA2 and the TNFB2 alleles, all developed multiple organ failure with fatal outcome.

Can we believe that this allele in IL-1Ra makes us more likely to be a patient with severe sepsis? What is known about this allele and IL-1Ra production? In one study, the amount of IL-1Ra produced from patients with insulin-dependent diabetes mellitus was reduced (Mandrup-Poulsen *et al*, 1994). In another study, granulocyte-macrophage colony stimulating factor was used to stimulate IL-1Ra production and persons with the IL-1RaA2 allele exhibited increased production compared with those without this allele (Danis *et al*, 1995b). In addition, in that study, there was reduced production of IL-1α in these subjects. Clearly, these results need to be confirmed but the concept that a gene polymorphism is associated with a measurable difference in the amount of the gene product and an outcome to disease may help resolve the problems encountered in new therapeutic clinical trials in septic shock patients. As it now stands, it seems that persons with the IL-1RaA2 allele make more IL-1Ra, which is a risk factor for developing severe sepsis following a surgical procedure.

Linkage of a particular cytokine gene polymorphism to disease is not always found in each population studied. For example, a predisposition to develop severe systemic lupus erythematosus was linked to the IL-1RaA2 allele in a cohort of persons in the U.K. (Blakemore *et al*, 1994). In a cohort living in Australia, this association was not found (Danis *et al*, 1995a). Similarly, the association of the IL-1Ra allele and ulcerative colitis in England (Mansfield *et al*, 1994) was not observed in a cohort of patients living in Southern Germany (Hacker *et al*, 1997). Therefore, the genetic studies in German patients need to be confirmed in another population.

NEW APPROACHES TO THERAPY

Although specific and successful monotherapy for a particular disease is a desirable goal in therapeutics, there are increasing examples where treatment is most effective when two or more agents are used. The obvious examples are cancer, autoimmune diseases, and HIV-1 where treatment with a single antitumor drug or immunosuppressive regimen or antiviral agent has been replaced with use of several agents, each targeting a somewhat different mechanism. If more than one agent would benefit the patient with septic shock, there are several possible combinations. In sepsis involving a Gram-negative organism, endotoxin itself acts like a cytokine in that it activates nearly the same genes as does IL-1 and TNF-α. A combination of neutralizing antibodies to endotoxin as well as blocking TNF and/or IL-1 may offer the patient with Gram-negative septic shock the greatest chance of rescue. Administration of anti-IL-8 with either anti-TNF or anti-IL-1 may be effective for patients with a high risk of developing ARDS. The concept of combining more than one anticytokine agent has sound experimental basis. For example, in animals, a combination of anti-TNF plus anti-IL-1 treatment has increased survival over that using either agent as monotherapy.

IS THE FAILURE TO RESCUE PATIENTS WITH ANTI-TNF OR IL-1 BLOCKADE DUE TO A DELAY IN INTERVENTION?

Whereas animal studies have provided a compelling argument that blocking TNF or IL-1 would be a therapeutic success in treating septic shock, in animals the window of time for reversing the events of lethal sepsis is rather small. Most studies pretreat animals. Does that mean that by the time the patient has overt signs of septic shock that it is too late for rescue with anticytokine therapy? In most of the anticytokine trials, the time that passes before a patient actually receives therapy can be 12 h after the randomization (Abraham *et al*, 1998). This can be days after the indications of altered mental status or blood pressure instability are observed. In one trial using monoclonal anti-TNF-α given within 12 h of the onset of severe sepsis, there was no change in the sequential samples of IL-1β, IL-6, or IL-1Ra despite anti-TNF-α intervention and no effect on physiologic abnormalities (Clark *et al*, 1998). The conclusion of the study was that there was inadequate neutralization of TNF-α, which was due to either insufficient dose or delayed administration.

What can be done to shorten the time between overt evidence of a life-threatening process and initiation of anticytokine therapy? In many patients (75%) with sepsis syndrome, blood pressure and organ perfusion are unimpaired; however, when septic shock develops, circulatory collapse within a few hours is thought to coincide with the onset of a new bacteremia or endotoxemia. In addition, a ''cytokine storm'' is thought to be responsible for triggering the shock. It has been the wisdom of anticytokine therapy that these patients also stand the greatest chance of a ''rescue'' by blocking further cytokine receptor triggering. In other patients with similarly serious infections, a fall in blood pressure or the development of organ failure is slower (over days) and cytokine receptors may have already been engaged before circulatory collapse reaches the level of entry criteria into a trial. In those patients, the administration of anticytokine therapy may be too late to provide a successful rescue.

As noted above, the heterogeneity of the acute infectious and underlying chronic disease processes in the patients who were enrolled into sepsis studies may have prevented demonstration of efficacy for anticytokine therapies. Additionally, large numbers of patients with low risk of mortality were enrolled in these clinical trials. Such patients often do not have markedly accelerated pro-inflammatory responses amenable to anticytokine therapy, and their inclusion in the clinical studies may have diluted out any survival benefit associated with the use of anticytokine therapy for sepsis. Therefore, an important advance for future trials would be to reduce patient heterogeneity. It has been the wisdom that larger patient cohorts, similar to the 20 000 patients used to evaluate thrombolytic therapy in acute myocardial infarction, would compensate for the ''background noise'' of patient heterogeneity in the sepsis trials; however, increasing the number of patients and the number of participating hospitals has yielded the same patient heterogeneity.

Nearly every sepsis trial has uncovered a subgroup defined retrospectively with survival benefit using the new therapy. When specific subgroups have been retested prospectively in follow-up larger trials, treating the same subgroup has eluted us in that patient heterogeneity in the expanded trials dilutes out the efficacy beyond statistical significance. Hence, anticytokine therapy for sepsis still awaits identification of the patient who can be rescued in a timely fashion from the downhill cascade caused by inflammatory cytokines.

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